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Cryptosporidium hominis n. sp. (Apicomplexa: Cryptosporidiidae) from *Homo sapiens*

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ABSTRACT. The structure and infectivity of the oocysts of a new species of *Cryptosporidium* from the feces of humans are described. Oocysts are structurally indistinguishable from those of *Cryptosporidium parvum*. Oocysts of the new species are passed fully sporulated, lack sporocysts, and measure 4.4–5.4 μm (mean = 4.86) × 4.4–5.9 μm (mean = 5.2 μm) with a length to width ratio 1.0–1.09 (mean 1.07) (n = 100). Oocysts were not infectious for ARC Swiss mice, nude mice, Wistar rat pups, puppies, kittens or calves, but were infectious to neonatal gnotobiotic pigs. Pathogenicity studies in the gnotobiotic pig model revealed significant differences in parasite-associated lesion distribution (P = 0.005 to P = 0.02) and intensity of infection (P = 0.04) between *C. parvum* and this newly described species from humans. In vitro cultivation studies have also revealed growth differences between the two species. Multi-locus analysis of numerous unlinked loci, including a preliminary sequence scan of the entire genome demonstrated this species to be distinct from *C. parvum* and also demonstrated a lack of recombination, providing further support for its species status. Based on biological and molecular data, this *Cryptosporidium* infecting the intestine of humans is proposed to be a new species *Cryptosporidium hominis* n. sp.

Key Words. "Cattle" genotype, *Cryptosporidium hominis* n. sp., *Cryptosporidium parvum*, "human" genotype, molecular analysis, new species, pathogenicity, taxonomy transmission studies.

IN 1912, E. E. Tyzzer described oocysts and the life-cycle of an unusual protozoan parasite that developed in the small intestine of old-world mice, and named this new species *Cryptosporidium parvum* (see Tyzzer 1912). Oocysts of *C. parvum* of the bovine genotype range in size from 5.0–5.5 × 3.7–5.0 μm with a mean size of 5.2 × 4.3 μm and a shape index of 1.20 (Fall et al. 2002). Other authors have reported a size range of 4.5–5.4 × 4.2–5.0 μm with a mean size of 5.0 × 4.5 μm and a shape index of 1.1 (Upton and Current 1985), and 4.7–6.0 × 4.4–5.0 μm with a mean size of 5.0 × 4.7 μm and a shape index of 1.06 (n = 50) (Fayer et al. 2001). Since Tyzzer's first description in 1912, oocysts corresponding in size and shape to *C. parvum* have been described in over 152 different host species, some of which have been named as separate species (Fayer et al. 2000).

The first two cases of human cryptosporidiosis were reported in 1976 (Meisel et al. 1976; Nime et al. 1976). Since then thousands of human cryptosporidial infections have been documented in 95 countries (see Casemore et al. 1997; Fayer et al. 2000; McLauchlin et al. 2000; Pedraza-Diaz et al. 2001).

Genetic analyses of oocysts recovered from humans however have indicated that most human cryptosporidial infections are caused by two distinct organisms previously referred to as the anthroponotic "human" genotype (genotype 1; genotype H) and the zoonotic "cattle" genotype (genotype 2; genotype C). Isoenzyme analysis, PCR-RFLP, and sequence analysis of a wide range of unlinked loci from different geographic locations have all demonstrated consistent genetic differences between the "cattle" genotype and the "human" genotype (Alves and Matos 2000; Alves et al. 2001; Awad-El-Kariem et al. 1995, 1998; Blears et al. 2000; Bonnin et al. 1996; Caccio et al. 1999, 2000; Carraway et al. 1996; Carreno et al. 2001; Gasser et al. 2001; Gibbons et al. 1998; Gobet and Toze 2001; Guyot et al. 2001; McLauchlin et al. 1999, 2000; Morgan and Thompson, 1998; Morgan et al. 1995, 1997, 1998a,b, 1999a,b,c,d, 2000a,b, 2001; Ogunkolade et al. 1993; Ong et al. 1999; Ortega et al. 1991; Patel et al. 1998; Pedraza-Diaz et al. 2001; Peng et al. 1997; 2001; Rochelle et al. 1999; Spano et al. 1997, 1998a,b,c; Sturbaum et al. 2001; Sulaiman et al. 1998, 1999, 2000, 2001, 2002; Vasquez et al. 1996; Widmer et al. 1998, 2000; Xiao et. al. 1998, 1999a,b,c, 2000; 2001a,b,c,d).

Bird (1981) named a *Cryptosporidium* isolate from a person with a severe immunodeficiency as *Cryptosporidium garnhami* based on the historical precedent that members of related genera (*Eimeria*, *Isospora*) were host specific, even though a previous morphological study of the same parasite showed no significant biologic differences between *C. parvum* and this parasite (Bird and Smith 1980). Because a detailed morphologic description was not given and differentiation characters were not provided in the publication that named *C. garnhami* as a new species (Bird 1981), this name must now be considered a nomen nudum. It is now known that immunodeficient patients are known

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to be infected with several *Cryptosporidium* parasites, including *C. parvum* human and cattle genotypes, *Cryptosporidium meleagridis*, *Cryptosporidium felis*, and *Cryptosporidium canis* (Morgan et al. 1999c, 2000b; Xiao et al. 2001d). Furthermore Bird (1981) provided no biological features with which to differentiate among these possible species.

Experimental transmission studies have shown that while the *C. parvum* "cattle" genotype is transmissible to a wide range of hosts, the "human" genotype is not infectious for mice or cattle (Casemore et al. 1997; Peng et al. 1997; Widmer et al. 1998, 2000). Recently, a neonatal pig model has been established for the human genotype (Widmer et al. 2000) and pathogenicity studies have revealed distinct biological differences between the "human" and "cattle" genotypes (Pereira et al. 2002). Based on these and additional studies provided below, we propose that the "human" genotype be considered a species separate from *C. parvum* and propose the name *Cryptosporidium hominis* n. sp.

MATERIALS AND METHODS

Oocysts for transmission studies. Four human-derived isolates from the Pathology Centre (isolates H138 and H151) and Murdoch Child Care Centre (isolates JAL 24 and JAL 54) in Perth, Western Australia, were used in transmission studies. All human isolates were confirmed as the human genotype by sequence analysis of the 18S rDNA and HSP-70 loci using previously described methods (Morgan et al. 1997, 2001). A cattle genotype isolate (S26) originally obtained from Switzerland and regularly passaged in the laboratory in mice was used as a positive control. All samples were purified using PBS-ether and Ficoll® density gradient centrifugation (Meloni and Thompson 1996). Excystation to ensure viability was carried out on all isolates using previously described methods (Meloni and Thompson 1996). Feces from all animals were examined by PCR and sequence analysis using the loci described above and also using malachite green, negative-staining microscopy (Elliot et al. 1999).

Oocyst measurements. *Cryptosporidium* oocysts (n = 100) from an AIDS patient in Peru (H2576) were measured with the aid of an ocular micrometer in a Zeiss Axioskop microscope at 1,000×.

Oocysts for pathogenicity studies. Human isolates used in pathogenicity studies in gnotobiotic pigs were obtained from AIDS patients in Peru (H2576 and H3438) and confirmed as the human genotype by sequence analysis of the 18S rDNA and HSP-70. Two cattle genotype isolates (GCH1 and OH) were also used as controls; *C. parvum* isolate GCH1 was graciously supplied to L. Ward by Saul Tzipori (Tufts University, School of Veterinary Medicine, Grafton, MA) and originally isolated from an AIDS patient. It has been maintained by passage in neonatal calves for several years. *Cryptosporidium parvum* isolate OH was obtained by L. Ward from an infected adult laboratory worker in Ohio and has been maintained by passage in neonatal calves. Both GCH1 and OH were confirmed as cattle genotypes by sequence analysis of the 18S rDNA and HSP-70.

Transmission studies. Animals were obtained from the Animal Resource Centres (which are specific pathogen-free breeding centres) at the various institutions. Feces of all animals were screened prior to infection using both PCR and microscopy. Negative controls were used in all experiments. Oocysts were quantified by hemacytometer and were administered by intubation using a plastic tube (diam. 0.7 mm) attached to a 1-ml syringe into the stomachs of six 1-day-old ARC Swiss mice, six 1-day-old nude mice, and six 1-day-old Wistar rat pups (Animal Resources Centre). Each animal received approximately 1×10^6 oocysts, less than three months old. The mice and rats were returned to their dams for 7 d and then euthanised by CO_2 exposure. Their gastrointestinal tracts, including jejunum, ileum, cecum, colon, and rectum were removed and processed as previously described (Meloni and Thompson 1996). Briefly, gastrointestinal tracts were minced and mucus removed by the addition of Sputasol in PBS (0.005 g/ml, Oxoid, Hampshire, England). Homogenates were purified using PBS-ether and Ficoll® density gradient centrifugation. Feces from both mice and rats were examined daily for the presence of *Cryptosporidium* oocysts using PCR and microscopy.

Six mixed-breed dogs supplied by the Animal Resources Centre at the University of Calgary that were two months old, with no history of *Cryptosporidium*, were inoculated with two human-derived isolates, each containing 8×10^5 oocysts in 10 ml PBS by oral gavage. Three dogs (2 female and 1 male) were inoculated with isolate H151 and the other three dogs (all male) were inoculated with isolate JAL24.

Similarly, six mixed-breed cats supplied by the Animal Resources Centre at the University of Calgary, also two months old with no history of *Cryptosporidium*, were inoculated with 8×10^5 oocysts in 2 ml PBS by oral gavage. Three cats (2 female and 1 male) were inoculated with isolate H151 and the other three cats (2 female and 1 male) were inoculated with isolate JAL24. Feces from both the dogs and cats were collected and screened daily for 16 d after inoculation.

Pathogenicity studies. Sixteen 1-day-old gnotobiotic pigs (four pigs per inoculum) were inoculated with 10^3 or 10^5 oocysts extracted from infected calf (GCH1, OH) or human (H2576, H3438) feces by immunomagnetic beads (DYNAL, Oslo, Norway). Two pigs that received each inoculum group were euthanised at 1 to 2 d post-onset of shedding and two pigs were euthanised at 7 to 10 d post-onset of shedding as detected by the acid fast-ultraviolet technique (Nielsen and Ward 1999). The small and large intestinal contents from each pig were collected and stored at 4 °C for further analysis. Sections of the cecum and colon, and upper, middle, and lower duodenum, jejunum, and ileum were collected and placed in Prefer® fixative for histologic examination.

Fixed tissues were processed through graded alcohols, embedded in paraffin, sectioned at 4 μm, placed on slides, and stained with hematoxylin and eosin (H&E). Slides were coded and examined without knowledge of infecting isolate and compared to uninfected age-matched controls. Intestinal infection rates per 100 linear μm of mucosal surface were evaluated at 40× magnification and scored 0 to 3 where 0 = no parasites detected, 1 = 1 to 10 parasites/100 linear μm, 2 = 11 to 30 parasites/100 linear μm, 3 = > 30 parasites/100 linear μm. Lymphoid hyperplasia was evaluated as the amount of inflammatory cells infiltrating in the submucosa/lamina propria and the size of organized submucosal lymphoid follicles (i.e. Peyer's patches), and scored 0 to 3, where 0 = same as age-matched controls, 1 = mild, 2 = moderate, 3 = marked. Mucosal attenuation was evaluated by villous height/width, crypt height/width, and degree of epithelial cytoplasmic vacuolation, and similarly scored 0 to 3 where 0 = same as controls, 1 = mild, 2 = moderate, 3 = severe. Chi-square (X^2) analysis was used to assess differences in the morphologic changes between groups. $P \leq 0.05$ was considered statistically significant.

RESULTS

Species description.

Cryptosporidium hominis n. sp.
(Fig. 1)

Description. Sporulated oocysts measure 4.4–5.4 μm (mean = 4.86) × 4.4–5.9 μm (mean = 5.2 μm) with a length to width

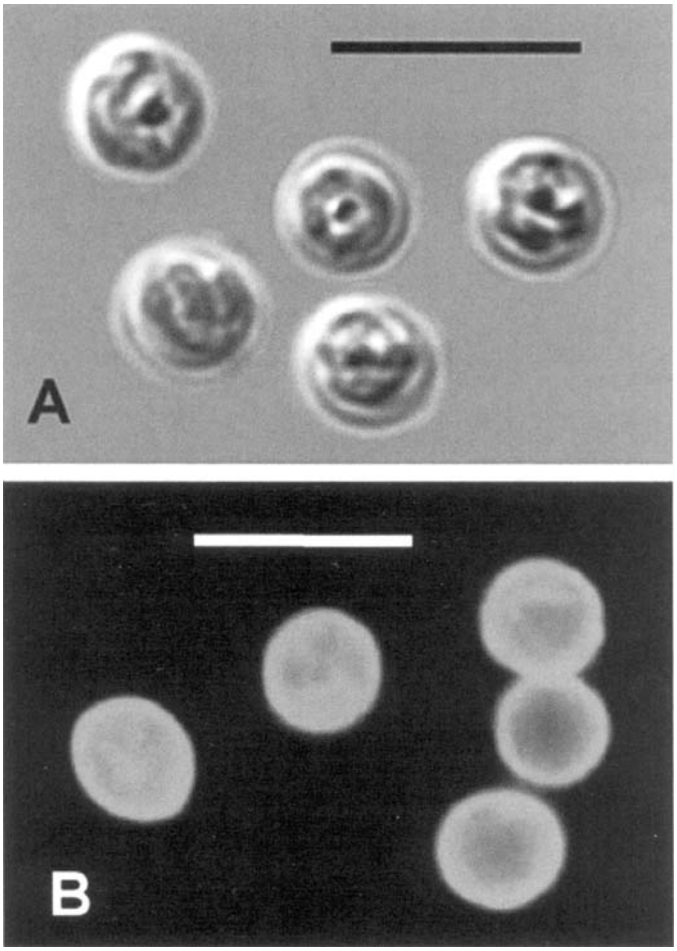

Fig. 1. Light micrographs of oocysts of *Cryptosporidium hominis* (isolate H2576) seen in differential interference contrast (**A**) and immunofluorescence (Meriflour) (**B**) microscopy. Bar = 10 μm.

ratio 1.0–1.09 (mean 1.07) (Isolate H2576). Four sporozoites are present in each oocyst (Fig. 1).

Type host. Humans, *Homo sapiens*.

Other hosts. Experimental infections have been produced in gnotobiotic pigs (Widmer et al. 2000) and a lamb (Giles et al. 2001). There has been one report of a natural infection in a dugong (*Dugong dugon*) (Morgan et al. 2000a) and one report of a natural infection in a lamb (Giles et al. 2002).

Type locality. Perth, Western Australia.

Other localities. Worldwide.

Location in host. Microvillous border of intestinal epithelial cells.

Prepatent period: Unknown.

Patent period: Unknown.

Sporulation time: Oocysts are passed fully sporulated.

Material deposited. A phototype of sporulated oocysts and GenBank accession numbers (see Table 2) have been deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland. USNPC no. 092045.

Etymology. This species is named *Cryptosporidium hominis* to reflect its host type.

Transmission studies. Microscopic examination of the purified gastro-intestinal tract from the *C. hominis*-inoculated mice, nude mice, and rats revealed no life-cycle stages of *C. hominis*. Oocysts were not detected in the feces of the mice, rats, and cats inoculated with *C. hominis*. These results were also confirmed using PCR analysis performed on feces and gut contents. However, neonatal rats, neonatal mice, and nude mice infected with *C. parvum* (cattle genotype) as the positive control all produced infections: oocysts were detected in both gut contents and feces (Table 1).

Inoculation of *C. hominis* into dogs did not produce an infection. However, one of the female dogs inoculated with the human-derived isolate H151 started to shed oocysts 7 d later and continued to shed intermittently for the next 8 d. A similar result occurred with a male dog inoculated with the human-derived isolate JAL24, that began to shed oocysts 4 d after inoculation and continued to shed them for the next six days. Genetic analysis of all these samples revealed that the dogs were shedding *C. canis* oocysts (data not shown). One explanation is that the two dogs had a latent *C. canis* infection that relapsed after inoculation with *C. hominis* oocysts, possibly from the stress of handling the dogs.

Pathogenicity studies. Microscopic examination of H&E-stained slides showed parasites throughout the length of the small and large intestine in the *C. parvum*-inoculated gnotobiotic pigs whereas parasites were observed only in the ileum and colon of all *C. hominis*-inoculated pigs. Significantly more parasites were observed per villus in the ileum of the *C. hominis*-inoculated pigs than in *C. parvum* inoculated pigs ($P = 0.004$). Moderate lymphoid hyperplasia with scattered inflammatory cell (neutrophils) infiltrates was observed in the duodenum at 1–2 d post-inoculation and in the jejunum, ileum, and colon at 7–10 d post-inoculation of the *C. parvum*-infected pigs whereas only mild lymphoid hyperplasia was observed in the ileum and colon of the *C. hominis*-infected pigs. Lymphoid hyperplasias in the duodenum ($P = 0.005$), jejunum ($P = 0.02$), ileum ($P = 0.02$), and colon ($P = 0.02$) were statistically different between the *C. parvum* and *C. hominis* isolates. Mild to moderate mucosal attenuation was observed throughout the small intestines and colons of the *C. parvum*-infected pigs whereas *C. hominis*-infected pigs showed only mild or no mucosal attenuation, which was restricted to the ileum and colon. Mucosal epithelial attenuation in the duodenum ($P = 0.005$), jejunum ($P = 0.02$), and ileum ($P = 0.02$) was also significantly greater in *C. parvum*-infected pigs than in those infected with *C. hominis*. Genotype analysis (data not shown) confirmed that pigs inoculated with *C. hominis* or *C. parvum* isolates had the same genotype in feces as in the inoculum.

Diagnosis. *Cryptosporidium hominis* is morphologically identical to *C. parvum* (cattle genotype). However, *C. hominis* can be differentiated from *C. parvum* on the basis of distinct genetic differences (Table 2), and the fact that *C. hominis*, unlike *C. parvum* is not transmissible to mice, nude mice, rats, cats nor dogs. In addition, pathogenicity studies in the gnotobiotic pig model revealed significant differences in parasite-associated lesion distribution ($P = 0.005$ to $P = 0.02$) and intensity of infection ($P = 0.04$) between *C. parvum* and this newly described species from humans (see discussion).

DISCUSSION

Cryptosporidium hominis n. sp. is commonly found to infect the small intestine and colon of humans (Casemore et al. 1997). The protozoan is widespread in the environment, and infection results from oral ingestion of oocysts encountered usually through fecal-oral contact or contaminated food or water (Arrowood 1997). Oocysts of *C. hominis* (5.0 × 4.2 μm, shape index, 1.19) overlap in size with *C. parvum* bovine genotype oocysts (5.2 × 4.3 μm, shape index 1.20) when measured using an Olympus Image Analysis system (Fall et al. 2002). However, morphology has been shown to be an unreliable method of de-

Table 1. Results of various recipient hosts inoculated with oocysts of *Cryptosporidium hominis* and *Cryptosporidium parvum*.

Isolate code	Species used for inoculations	Age and strain of recipient host	No of animals infected	Oocyst dose per recipient	Feces[a]	Gut examination[a]	PCR[c]	Location of study[c]
S26	*C. parvum*	6-day ARC/Swiss mouse	7	1×10^6	4+	4+	*C. parvum*	Mu
		6-day ARC/nude mouse	6	1×10^6	4+	4+	*C. parvum*	Mu
		6-day ARC/Wistar rat pups	8	1×10^6	4+	4+	*C. parvum*	Mu
H151	*C. hominis*	6-day ARC/Swiss mouse	10	1×10^6	—[d]	—	—	Mu
		6-day ARC/nude mouse	8	1×10^6	—	—	—	Mu
		6-day ARC/Wistar rat pups	9	1×10^6	—	—	—	Mu
		60 day old dog pups	3	1×10^5	3+	3+	*C. canis*	UCa
		60 day old kittens	3	1×10^5	—	—	—	UCa
H138	*C. hominis*	6-day ARC/Swiss mouse	7	1×10^6	—	—	—	Mu
		6-day ARC/nude mouse	9	1×10^6	—	—	—	Mu
		6-day ARC/Wistar rat pups	6	1×10^6	—	—	—	Mu
UP JAL 24	*C. hominis*	6-day ARC/Swiss mouse	9	1×10^6	—	—	—	Mu
		6-day ARC/nude mouse	7	1×10^6	—	—	—	Mu
		6-day ARC/Wistar rat pups	8	1×10^6	—	—	—	Mu
		60 day old dog pups	3	1×10^5	3+	3+	*C. canis*	UCa
		60 day old kittens	3	1×10^5	—	—	—	UCa
UP JAL 54	*C. hominis*	6-day ARC/Swiss mouse	9	1×10^6	—	—	—	Mu
		6-day ARC/nude mouse	8	1×10^6	—	—	—	Mu
		6-day ARC/Wistar rat pups	7	1×10^6	—	—	—	Mu

[a] = semi-quantitative scoring method whereby 3+ = > 2×10^5/ml and 4+ > 2×10^6/ml.
[b] = samples were amplified by the polymerase chain reaction and sequenced to confirm species.
[c] ; Mu = Murdoch University; Uca = University of Calgary
[d] - = not detected.

limiting species within the genus *Cryptosporidium* (Fall et al. 2002; Fayer et al. 2001).

Like the present study, most studies have found that *C. hominis* oocysts were not infective for mice or cattle (Fayer, pers. commun.; Giles et al. 2001; Peng et al. 1997; Widmer et al. 1998; 2000). The results of this study also demonstrate that *C. hominis* is not infective to mice, rats, cats nor dogs. A gnotobiotic pig model has been established for *C. hominis* (Pereira et al. 2002; Widmer et al. 2000) and there have been two reports of non-human infections with *C. hominis*. One report identified *C. hominis* in a dugong (*Dugong dugon*) (Morgan et al. 2000a) and another reported *C. hominis* in a lamb (Giles et al. 2001). However, it was not possible to determine the immune status of these hosts and all other reports of *C. hominis* infections have been confined to humans and primates (Xiao et al. 1999c). In contrast, *C. parvum* is known to cause widespread infection in humans and various ruminants under natural conditions, and mice, rats, and dogs in experimental infections (Fayer et al. 2000; cf. O'Donoghue 1995). It is possible that some species of animals can be infected with *C. hominis* under certain circumstances, but the evidence so far has clearly demonstrated that the spectrum of host specificity of *C. hominis* is far more restricted than that of *C. parvum*.

A recent study, which exploited the gnotobiotic pig model, has shown differences in pathogenesis between the two species when they infect the same host. Pereira et al. (2002) reported that the prepatent period was longer for *C. hominis* than for *C. parvum* (8.8 vs. 5.4 d), and that *C. parvum*-infected pigs developed moderate to severe diarrhea and dehydration whereas *C. hominis*-infected pigs had mild to moderate diarrhea without dehydration. In addition, parasites were seen microscopically in the intestines of the *C. parvum*-infected pigs during the prepatent and patent periods whereas parasites were seen microscopically only during the patent period in *C. hominis*-infected pigs. Also, as observed in the present study, parasites were seen microscopically throughout the small and large intestine of the *C. parvum*-infected pigs but were observed only in the ileum and colon of the *C. hominis*-infected pigs where *C. hominis* had higher numbers of parasites per villus. Furthermore, moderate to severe villous/mucosal attenuation with marked lymphoid hyperplasia was seen throughout the intestines of *C. parvum*-infected pigs whereas lesions in *C. hominis*-infected pigs were mild to moderate and restricted to the ileum and colon (Pereira et al., 2002).

Cryptosporidium hominis and *C. parvum* also demonstrate different biological activity in culture (Hijjawi et al. 2001). A recent study has shown that *C. hominis* grew much more aggressively than *C. parvum* in HCT-8 cells. After 72 h post-infection with oocysts of *C. hominis*, HCT-8 cells were infected with large numbers of parasitic stages, especially merozoites that could be seen continuously attempting to penetrate the cells. Furthermore, *C. hominis* completed its life cycle with the production of oocysts within 72 h whereas many fewer stages were seen and oocysts of *C. parvum* could not be detected in culture until five days post-infection (Hijjawi et al. 2001). These findings are in contrast to the gnotobiotic pig model, wherein the prepatent period was longer for *C. hominis* than for *C. parvum* (8.8 vs. 5.4 d). The difference may be due to the fact that *C. hominis* may be better adapted to the human epithelial cell line used for in vitro studies (HCT-8), whereas *C. parvum* may be more adapted to the pig.

Molecular and phylogenetic analysis of numerous unlinked loci supports the species status of *C. hominis* (see Table 2). Phylogenetic analysis of *C. hominis* and *C. parvum* at the 18S rRNA locus revealed a similarity of 99.7% (Morgan et al. 1999a). The similarity between *Toxoplasma gondii* and *Neospora caninum* is 99.8% at this locus. At the ITS locus, the similarity between *C. hominis* and *C. parvum* is only 82.23%. The similarity between *T. gondii* and *N. caninum* at this locus is 89.49% (Morgan et al. 1999d). Phylogenetic analysis at the HSP-70 locus produced similar results with a genetic similarity between *C. hominis* and *C. parvum* of 98.50% (Sulaiman et al. 2000). At the dihydrofolate reductase-thymidylate synthase (DHFR) gene, the genetic similarity between *C. parvum* and *C.*

Table 2. Loci used to differentiate between *Cyptosporidium hominis* and *Cryptosporidium parvum*.

Loci examined	GenBank accession numbers	Reference
AcetylCoA	U24082, AF102766, AF102767	Morgan et al. 1998b
Actin Gene	AF382338, AF382337	Sulaiman et al. 2002
β-tubulin	Y12615, AF115399, AF115398, AF323580, AF323579, AF323578, AF323576, AF074936, AF074935	Caccio et al. 1999; Rochelle et al. 1999; Sulaiman et al. 1999; Widmer et al. 1999; Perez & Le Blanq, 2001
COWP	AF266273, AF266265, AF248743, AF248741	Spano et al. 1997; Patel et al. 1999; McLauchlin et al. 1999; 2000; Xiao et al. 2000; Giles et al. 2001; Pedraza-Diaz et al. 2001
Double-stranded RNA virus	AF169922–AF169929, AF169934–AF169940	Khramtsov et al. 2000; Xiao et al. 2001a
Dihydrofolate reductase thymidylate synthase gene (DHFR).	U41366, U41365	Vasquez et al. 1996; Morgan et al. 1999b; Gibbons et al. 1998
GP60	AF403177, AF403176, AF403175, AF403174	Strong et al. 2000; Peng et al. 2001
GP40/15 gene	AF178696, AF178695, AF178694, AF178692, AF178691, AF224464, AF178693, AF224463, AY048667, AY048666, AYO48665	Strong et al. 2000; O'Connor et al. 2002
Heat shock gene (HSP70)	U11761, AF221528, AF221535	Sulaiman et al. 2000; Gasser et al. 2001; Gobet & Toze, 2001; Straub et al. 2002
18S rRNA	AF093493, AF093492, AF093491, AF093490, AF093489, AF164102, AF308600, AF248748, AF248747, AF108865, AF108864, AF015772	Le Blancq et al. 1997; Morgan et al. 1997; McLauchlin et al. 1999; Morgan et al. 1998a; 1998b; 1999b; Patel et al. 1999; Lowery et al. 2000; Morgan et al. 2000a; 2000b; Xiao et al. 2000; 2001c; 2001d; Alves et al. 2001; Lowery et al. 2001a; Pedraza-Diaz et al. 2001; Guyot et al. 2001; Tiangtip & Jongwutiwes, 2002
ITS1, 5.8S, ITS2	AF015773, AF093012, AF093008	Carraway et al. 1996; Le Blancq et al. 1997; Morgan et al. 1999a; Ong et al. 1999
Microsatellite loci	AJ308567, AJ308566, AJ308565, AJ308564, AF344880, AJ249587, AJ249586, AJ249585, AJ249584	Caccio et al. 2000; Feng et al. 2001
Poly-threonine gene	U83169	Carraway et al. 1996; Yagita et al. 2001; Spano et al. 1997
Ribonucleotide reductase (RNR)	AF275635, AF043243	Spano et al. 1997; Widmer et al. 1998
TRAPC1	AF033829, AF033828, X77587, AF017267	Spano et al. 1998a; 1998b; McLauchlin et al. 1999
TRAPC2	X77586, AF082524, AF082523, AF082521	Peng et al. 1997; Sulaiman et al. 1998; Lowery et al. 2001b; 2001b; McLauchlin et al. 1999
Cryptosporidium genome project (*C. parvum* + *C. hominis*)	http://www.cbc.umn.edu/ResearchProjects/AGAC/Cp/index.htm http://www.parvum.mic.vcu.edu/	

hominis was 97%. A preliminary survey of the *C. hominis* genome covering approximately 2% of the genome and encompassing 200 kb of unique sequence showed an average similarity of approximately 95% between *C. hominis* and *C. parvum* (Widmer et al. 2000). Another study reported that *C. parvum* constitutively expresses 2 types of rRNA genes (Type-A and Type B; Le Bancq et al. 1997), whereas more than two transcripts were detected in *C. hominis* (Xiao et al. 1999a). Fundamental differences in ribosomal gene expression between *C. hominis* and *C. parvum* emphasize significant differences between the two species (Widmer et al. 1999).

Despite the large number of isolates examined at multiple unlinked loci from a wide range of geographic locations, putative recombinants between *C. hominis* and *C. parvum* have never been identified. Since both species infect humans and mixed infections in humans have been documented (Carraway et al. 1996; McLauchlin et al. 1998; Morgan et al., unpubl., Patel et al. 1998), this lack of recombination is not due to a physical separation into different host species, rather it indicates reproductive isolation between *C. hominis* and *C. parvum*.

It is difficult to assess the duration of diarrhea or severity of infection with *C. hominis* because human volunteer trials have tested only the bovine genotype of *C. parvum* (Messner et al. 2001; Okhuysen et al. 1999) and, most previous clinical reports of cryptosporidiosis in humans did not indicate the genotype of the parasite. There do, however, appear to be other distinct biological differences between *C. hominis* and *C. parvum*. A multilocus study in the United Kingdom examined 211 samples *Cryptosporidium*-positive fecal samples from humans using molecular methods and reported that *C. hominis* was detected in a significantly greater proportion of the samples with larger numbers of oocysts whereas *C. parvum* (bovine genotype) was detected in a significantly greater proportion of the samples with small numbers of oocysts. There were no significant dif-

ferences in the distribution of *C. hominis* or *C. parvum* by patient sex and age (McLauchlin et al. 1999). A molecular epidemiological study of *Cryptosporidium* in children in Lima, Peru, reported that the duration of oocyst detection in stool was significantly longer and the intensity of oocyst shedding was significantly higher for infections with *C. hominis* than for infections with *C. parvum* (Xiao et al. 2001d). These findings suggest that there are distinct differences in oocyst shedding patterns between these two species.

The correct identification of *Cryptosporidium* species in clinical and epidemiological studies has important public health implications. A recent study in the UK, which examined 1,705 fecal samples from humans, reported distinct geographical and temporal variations in the distribution of *C. parvum* and *C. hominis* infections in patients (McLauchlin et al. 2000). *Cryptosporidium parvum* was more common during spring whereas *C. hominis* was significantly more common in patients infected during late summer-autumn in those with a history of foreign travel (McLauchlin et al. 2000). Most European studies have reported that *C. parvum* is more common in patients than *C. hominis* (Alves et al. 2001; Guyot et al. 2001, McLauchlin et al. 1999; Pedraza-Diaz et al. 2001), whereas studies in Australia, the USA, and South America have reported *C. hominis* to be the most common *Cryptosporidium* species infecting humans (Morgan et al. 1998a; Sulaiman et al. 1998; Widmer at al. 2000; Xiao et al. 1999b, 2001d). Molecular data combined with biological differences in host occurrence and pathogenicity indicate that *C. hominis* is a distinct species.

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